

Systematic significance of leaf epidermal features in *Apios* and *Cochlianthus* (Leguminosae)

^{1,2}REN Bo ¹ZHU Xiang-Yun* ³JIANG Yan-Cheng*

¹ (Institute of Botany, the Chinese Academy of Sciences, Beijing 100093, China)

² (Graduate University of Chinese Academy of Sciences, Beijing 100049, China)

³ (College of Biology and Technology, Xinjiang University, Xinjiang 830046, China)

Abstract Nineteen leaf epidermis samples representing six species in *Apios* and two in *Cochlianthus* were examined for the first time using both light microscopy and scanning electron microscopy. Leaf epidermal characters of these two closely related genera, such as shape of cells, pattern of anticlinal walls, cuticular membrane and wax ornamentation, are usually stable within species and thus of great significance in understanding the relationships between and within genera. The results indicate that (1) *A. gracillima* Dunn should be recognized as a distinct species, not a variety of *Apios delavayi* Franch., (2) the division of *Apios* into two subgenera is unreasonable, and (3) *Apios* and *Cochlianthus* are retained as two distinct genera.

Key word Leguminosae, *Apios*, *Cochlianthus*, leaf epidermis, systematics.

The genera *Apios* Fabr. and *Cochlianthus* Benth. are members of subtribe Erythrinae, tribe Phaseoleae in the subfamily Papilionoideae of Leguminosae. *Apios* is distributed in East Asia and North America, consisting of no more than 10 species, and *Cochlianthus*, which is restricted to East Asia, has only two distinct species. These two genera have long been considered to be very closely related (Li, 1952; Hutchinson, 1964; Lackey, 1981; Woods, 1988; Li, 1995).

Apios has great economic importance. It has been used as food because of its high dry weight protein content and genistein, an anticarcinogenic compound, found in its edible tubers (Safford, 1925; Haskin, 1929; Walker, 1939; Duke, 1984; Blackmon, 1986; Vietmeyer, 1986; Walter et al., 1986; Krishnan, 1998). Extensive research has been done on the genus, including studies on reproduction (Bruneau & Anderson, 1988), pollination (Westerkamp & Paul, 1993; Bruneau & Anderson, 1994), cytology (Atchison, 1949; Lewis et al., 1962; Seabrook & Dionne, 1976) and evolution (Joly & Bruneau, 2004). The genus *Cochlianthus* has rarely been studied. However, there are few taxonomic revisions of these two genera, other than that of Woods (1988), whose work was mainly based on gross-morphology.

Leaf epidermal features have a number of advantages as taxonomic markers. They are of value not only in making taxonomic decisions but also in developmental and evolutionary studies (Stace, 1984). The leaf epidermal features in *Apios* and *Cochlianthus*, however, have not yet been examined. The purpose of this study is to find significant taxonomic characters of the leaf epidermis for a better understanding of the relationship and positions of the two genera in tribe Phaseoleae.

1 Material and methods

Mature leaves of 19 specimens representing six species in *Apios* and two in *Cochlianthus* were collected from PE, E and GH (Table 1). The leaves for light microscopy (LM) study were boiled in water before maceration in 20% NaClO solution. Pieces of leaf epidermis were stained in a solution of 1% safranin (in 50% alcohol) before being mounted in gum. To check the constancy of epidermal structure, three leaves were collected for each species, and at least three slides were made from different parts of a single leaf. The stomatal index (I) was calculated using the formula $I=S/(E+S)\times 100$, where S denotes the number of stomata per unit area and E the number of epidermal cells of the same area. Materials for scanning electron microscopy (SEM) observation were directly mounted on stubs without any treatment. After gold-sputtering, the specimens were examined and photographed under a Hitachi S-800.

The terminology followed that of Wilkinson (1979) and of Dilcher (1974).

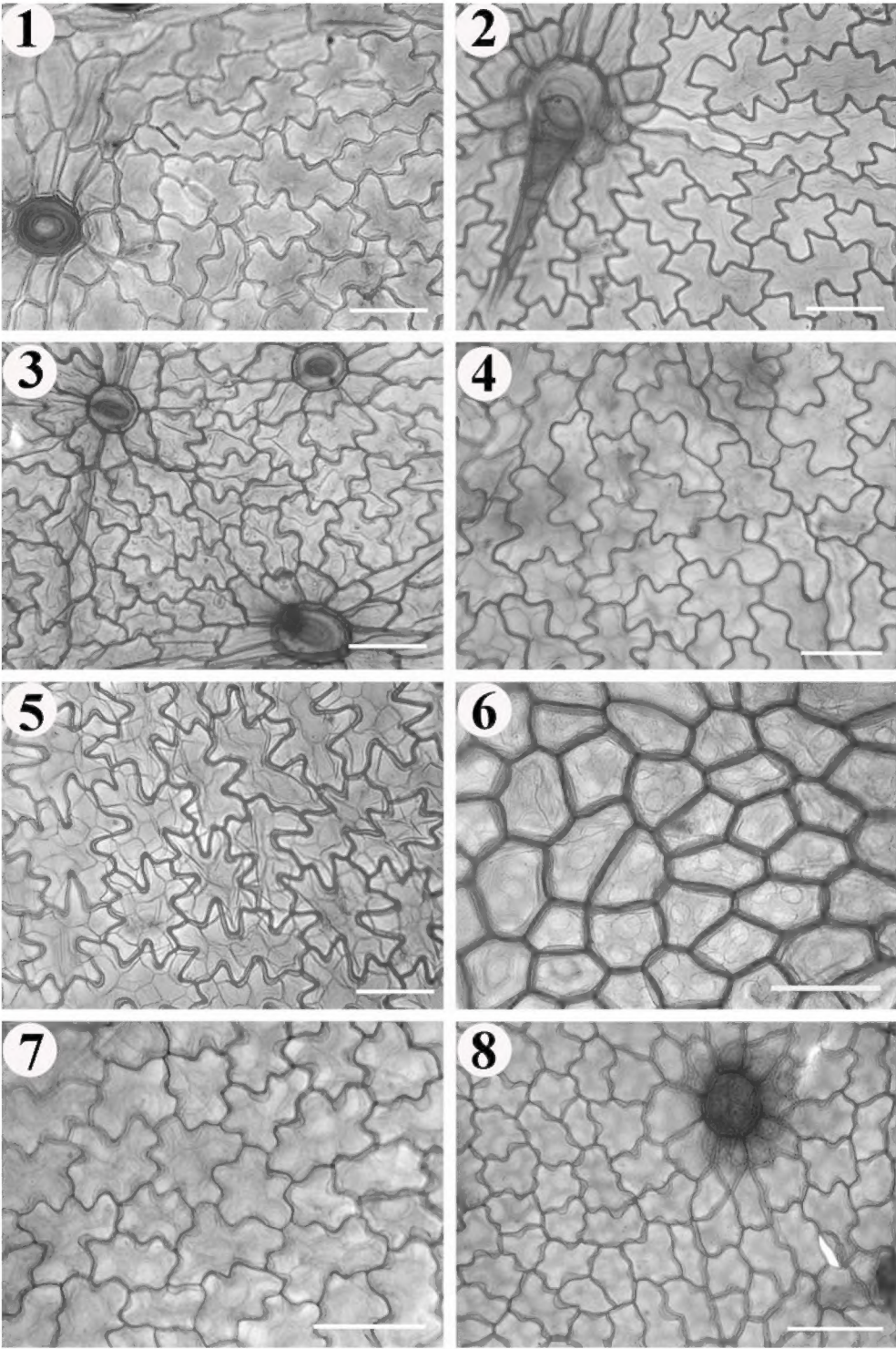
Table 1 Source of materials

Species	Locality	Voucher
<i>Apios americana</i> Medik.	Massachusetts, U.S.A.	V. Bates & I. S. Elsik 218 (PE)
	Maine, U.S.A.	J. C. Solomon & A. Solomon 20056 (PE)
	Florida, U.S.A.	J. S. Miller & M. C. Merello 9063 (PE)
<i>A. priceana</i> B. L. Rob.	Mississippi, U.S.A.	J. D. Ray 6728 (GH)
	Tennessee, U.S.A.	H. K. Svenson 7325 (GH)
<i>A. carnea</i> (Wall.) Benth. ex Baker	Zhongdian, Yunnan, China	B. Ren 80 (PE)
	Bomi, Xizang, China	B. S. Li & C. C. Ni 6855 (PE)
	Fengjie, Sichuan, China	Z. R. Zhang 25930 (PE)
<i>A. delavayi</i> Franch.	Zhongdian, Yunnan, China	B. Ren 78 (PE)
	Zayü, Xizang, China	C. C. Ni 180 (PE)
	Kangding, Sichuan, China	K. Y. Lang & L. Q. Li 1250 (PE)
<i>A. fortunei</i> Maxim.	Without precise locality, Anhui, China	Y. L. Kang 950 (PE)
	Hangzhou, Zhejiang, China	S. Y. Zhang 1402 (PE)
	Xianyou, Fujian, China	Y. Lin 437 (PE)
<i>A. gracillima</i> Dunn	Dongchuan, Yunnan, China	H. D. McLaren L111 (E)
	Lijiang, Yunnan, China	E. E. Maire 731 (E)
<i>Cochlianthus gracilis</i> Benth.	Cona, Xizang, China	Qinghai-Xizang Exped. 2444 (PE)
	Cona, Xizang, China	Qinghai-Xizang Exped. Supplement Team 751695 (PE)
<i>C. montanus</i> (Diels) Harms	Zayü, Xizang, China	G. Forrest 6108 (PE)

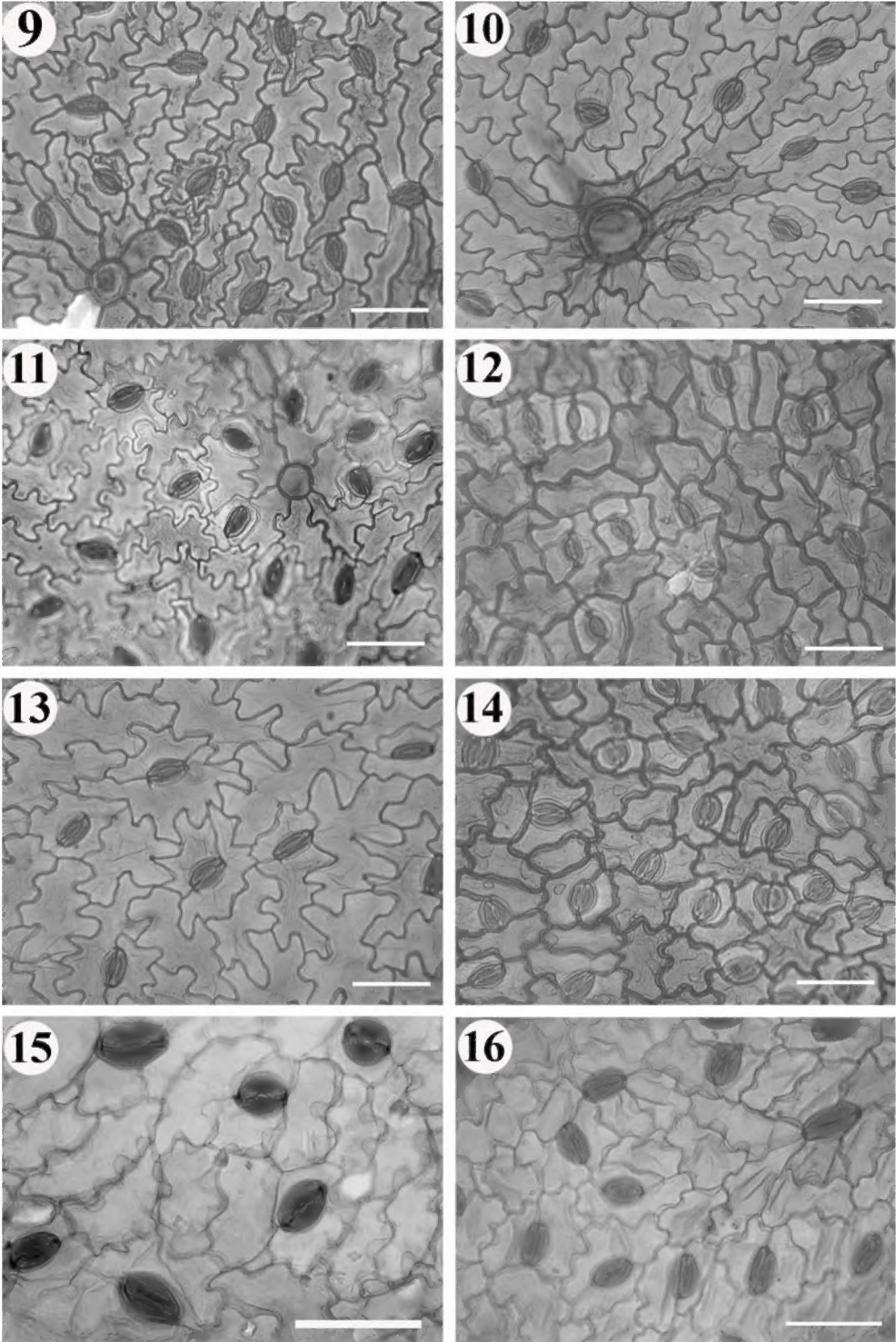
2 Results

2.1 Characteristics of leaf epidermis under LM

Hairs were abundant on both adaxial and abaxial epidermis of the two species of *Cochlianthus*, but only present slightly on the veins and veinlets of the six species of *Apios*. In most species, the epidermal cells were irregular in outline, with sinuolate (Figs. 7, 8, 12, 15, 16) or sinuate (Figs. 1–5, 9–11, 13) anticlinal walls, and the shapes of epidermal cells of a same species were similar on both adaxial and abaxial sides. In only one species, *Apios gracillima*, the shape of adaxial epidermal cells was polygonal with straight to arched anticlinal walls and the abaxial epidermal cells were irregular with sinuolate anticlinal walls



Figs. 1–8. Characteristics of adaxial epidermal cells in *Apios* and *Cochlianthus* under LM. 1. *A. americana*. 2. *A. priceana*. 3. *A. carnea*. 4. *A. delavayi*. 5. *A. fortunei*. 6. *A. gracillima*. 7. *Cochlianthus gracilis*. 8. *C. montanus*. Scale bar = 50 μm .



Figs. 9–16. Characteristics of abaxial epidermal cells in *Apios* and *Cochlianthus* under LM, type of stomatal apparatus. **9.** Anomocytic of *A. americana*. **10, 11, 13.** Laterocytic. **12, 14–16.** Paracytic. **10.** *A. priceana*. **11.** *A. carnea*. **12.** *A. delavayi*. **13.** *A. fortunei*. **14.** *A. gracillima*. **15.** *C. gracilis*. **16.** *C. montanus*. Scale bar=50 μm.

(Figs. 6, 14). Stomata appeared only on the abaxial epidermis of all species. The stomatal apparatuses were anomocytic (*A. americana*, Fig. 9), laterocytic (*A. priceana*, *A. carnea*, *A. fortunei*, Figs. 10, 11, 13), or paracytic (*A. delavayi*, *A. gracillima*, *C. gracilis*, *C. montanus*, Figs. 12, 14–16), with anisocytic stomata occasionally observed in some species (*A. priceana*, *A. delavayi*, *A. gracillima*, *C. montanus*, Figs. 10, 12, 14, 16) (Table 2). The stomatal index measured on these eight species ranged between 16.1 and 26.3 (Table 2).

Table 2 The characters of leaf epidermis in *Apios* and *Cochlianthus* under LM

Species	Adaxial epidermis		Abaxial epidermis		Size of stomata (μm ²)	Stomatal index	Stomatal type	Other types of stomatal apparatus occasionally observed
	Shape of cells	Pattern of anticlinal walls	Shape of cells	Pattern of anticlinal walls				
<i>Apios americana</i>	Irr	Sin	Irr	Sin	24.6×14.8	26.3	Ano	Lat
<i>A. priceana</i>	Irr	Sin	Irr	Sin	23.6×16.2	19.2	Lat	Ani
<i>A. carnea</i>	Irr	Sin	Irr	Sin	23.8×14.8	23.0	Lat	Ano
<i>A. delavayi</i>	Irr	Sin	Irr	Si	23.4×17.6	19.4	Par	Ani, Ano
<i>A. fortunei</i>	Irr	Sin	Irr	Sin	28.0×17.8	18.9	Lat	–
<i>A. gracillima</i>	Pol	Str-arc	Irr	Si	25.0×19.2	24.0	Par	Ani
<i>Cochlianthus gracilis</i>	Irr	Si	Irr	Si	26.2×20.4	15.1	Par	Ano
<i>C. montanus</i>	Irr	Si	Irr	Si	21.4×12.4	19.5	Par	Ani, Ano

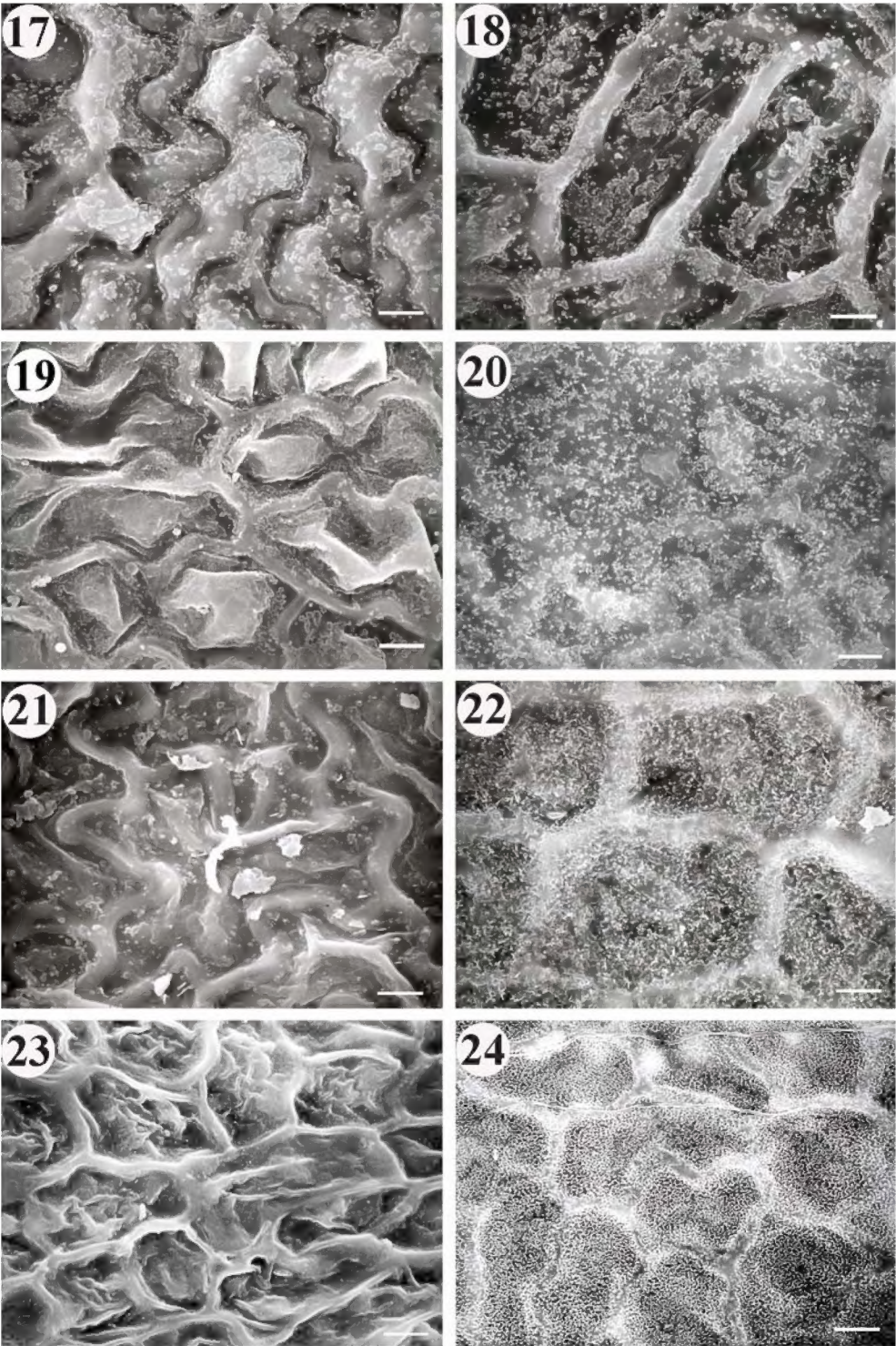
Ani, anisocytic; Ano, anomocytic; Irr, irregular; Lat, laterocytic; Par, paracytic; Pol, polygonal; Si, sinuolate; Sin, sinuate; Str-arc, straight to arched.

2.2 Characteristics of leaf epidermis under SEM

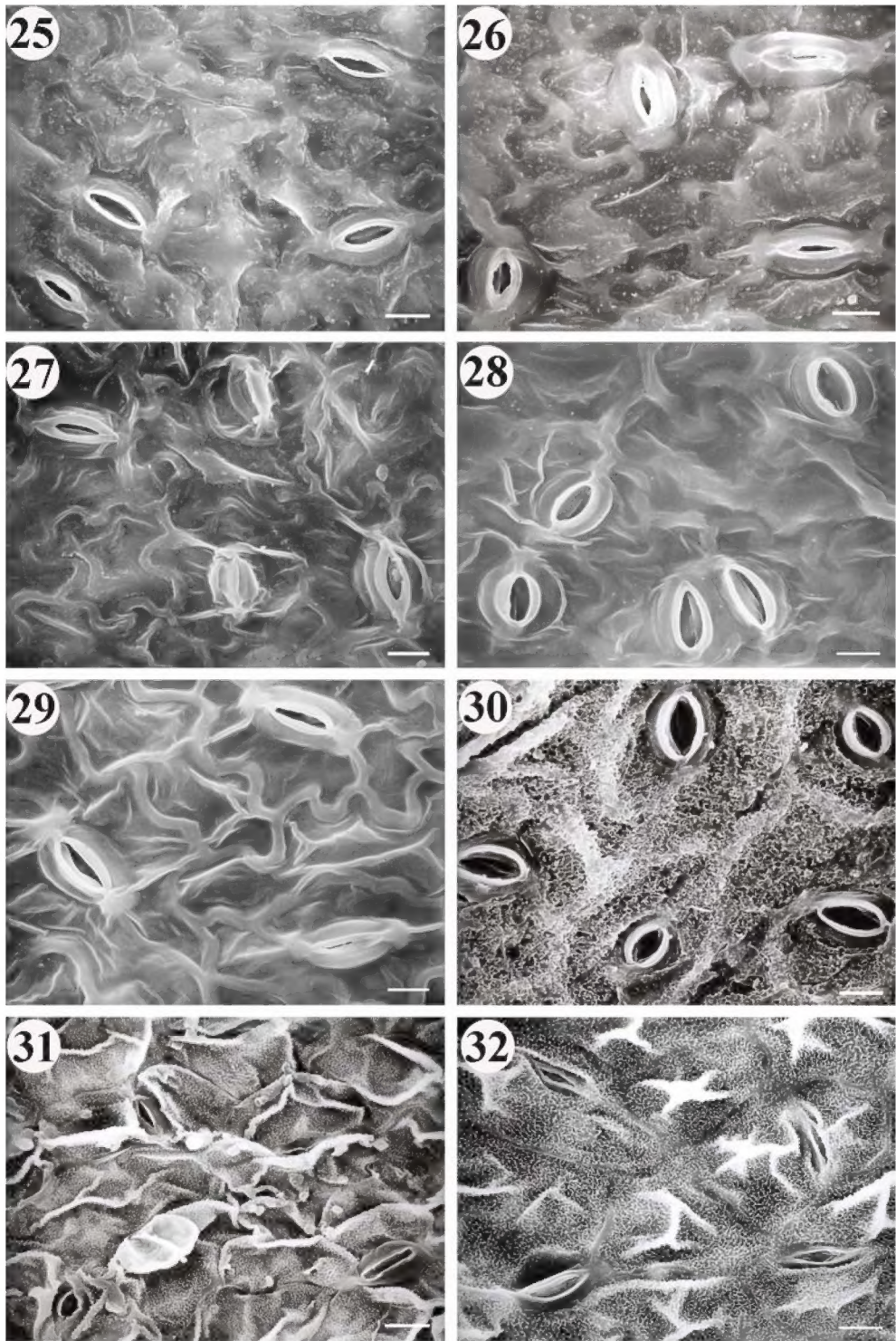
The cuticular membrane was smooth (Figs. 20, 22, 24, 30), striate (Figs. 17, 18, 21, 26–29), ridged (Figs. 19, 25, 31, 32) or reticulate (Fig. 23). The wax ornamentation, if present, was flake-like (Figs. 20, 22, 24, 30–32), scale-like (Figs. 17–19, 25) or granular (Figs. 21, 26). The inner margin of the outer stomatal rim was smooth in *Apios carnea* (Fig. 35) and *A. fortunei* (Fig. 37), and undulate in the other species (Figs. 33, 34, 36, 38–40) (Table 3).

Table 3 The characters of leaf epidermis in *Apios* and *Cochlianthus* under SEM

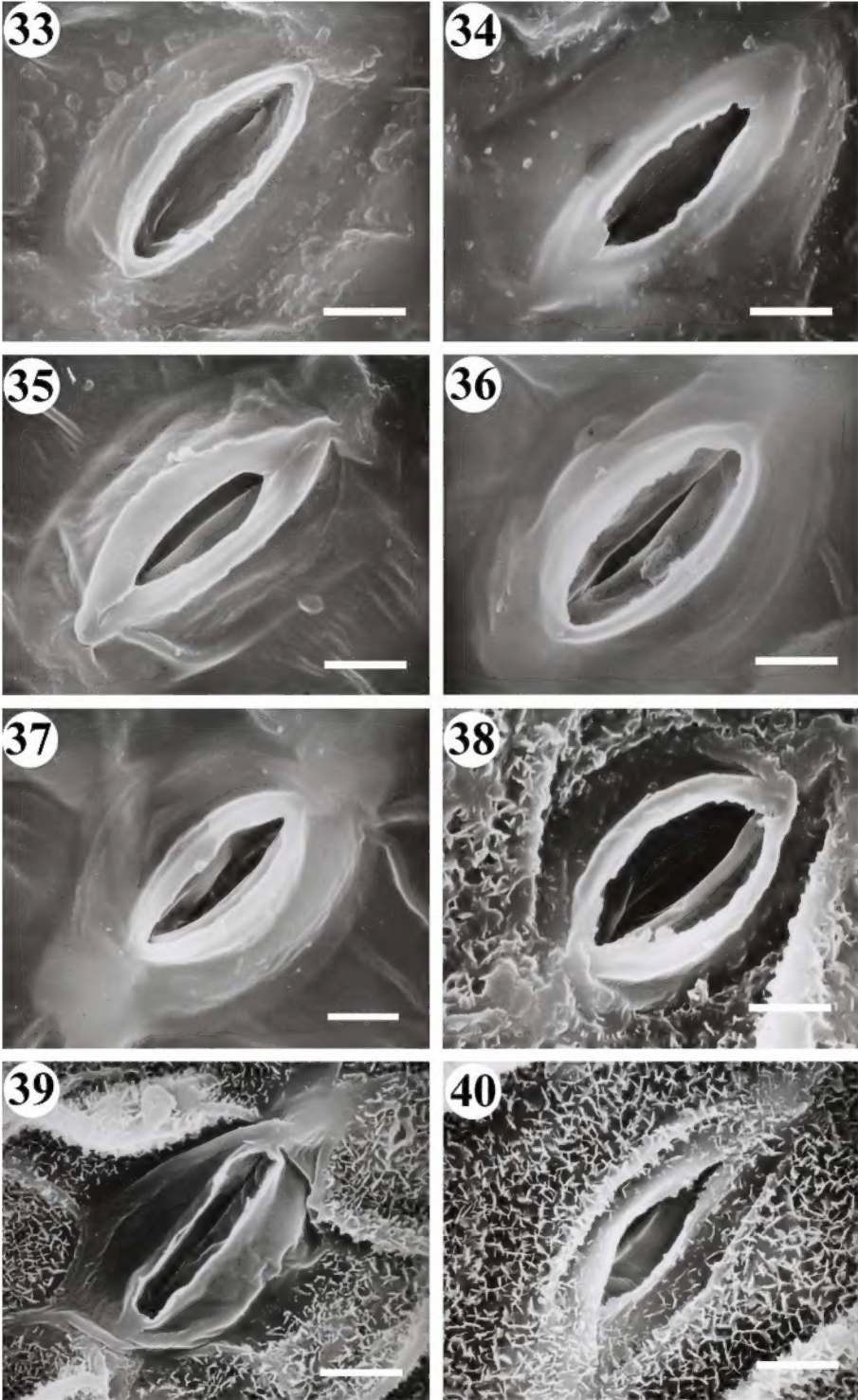
Species	Adaxial epidermis		Abaxial epidermis		Inner margin of outer stomatal rim
	Cuticular membrane	Wax ornamentation	Cuticular membrane	Wax ornamentation	
<i>Apios americana</i>	striate	scale-like	ridged	scale-like	undulate
<i>A. priceana</i>	striate	scale-like	striate	granular	undulate
<i>A. carnea</i>	ridged	scale-like	striate	invisible	smooth
<i>A. delavayi</i>	smooth	flake-like	striate	invisible	undulate
<i>A. fortunei</i>	striate	granular	striate	invisible	smooth
<i>A. gracillima</i>	smooth	flake-like	smooth	flake-like	undulate
<i>Cochlianthus gracilis</i>	reticulate	invisible	ridged	flake-like	undulate
<i>C. montanus</i>	smooth	flake-like	ridged	flake-like	undulate



Figs. 17–24. Characteristics of adaxial epidermal cells in *Apios* and *Cochlianthus* under SEM. 17. *A. americana*. 18. *A. priceana*. 19. *A. carnea*. 20. *A. delavayi*. 21. *A. fortunei*. 22. *A. gracillima*. 23. *C. gracilis*. 24. *C. montanus*. Scale bar=10 μ m.



Figs. 25–32. Characteristics of abaxial epidermal cells in *Apios* and *Cochlianthus* under SEM. 25. *A. americana*. 26. *A. priceana*. 27. *A. carnea*. 28. *A. delavayi*. 29. *A. fortunei*. 30. *A. gracillima*. 31. *C. gracilis*. 32. *C. montanus*. Scale bar=10 μ m.



Figs. 33–40. Characteristics of stomatal apparatus in *Apios* and *Cochlianthus* under SEM. 33. *A. americana*. 34. *A. priceana*. 35. *A. carnea*. 36. *A. delavayi*. 37. *A. fortunei*. 38. *A. gracillima*. 39. *C. gracilis*. 40. *C. montanus*. Scale bar=10 μ m.

3 Discussion

No consensus has been reached on the number of species in *Apios* (Li, 1952; Hutchinson, 1964; Lackey, 1981; Woods, 1988; Li, 1995). Since de Candolle (1825), there has only been one worldwide revision of *Apios* by Woods (1988), who recognized five species in this genus: *A. americana*, *A. carnea*, *A. delavayi* (including *A. delavayi* var. *gracillima*), *A. fortunei* and *A. priceana*. After observation about sixty specimens of *A. gracillima* and *A. delavayi*, Woods believed that they are not different in growth habit, leaflet size, pedicel length and inflorescence, and the only difference between them is that the flower of *A. gracillima* is purple while the *A. delavayi* is greenish. In his opinion, *A. gracillima* does not merit recognition as a distinct species, but should be treated as a variety of *A. delavayi*.

From 200 specimens examined, we have found some distinct and stable features between *A. gracillima* and *A. delavayi*: the flower in *A. gracillima* is from red to purple and the leaflets are obtuse at apex with a small mucro, while in *A. delavayi*, the flower is from yellow-green to pale violet and the leaflets are acuminate at apex. This study shows that the leaf epidermal characters of these two taxa are also very distinct: on the adaxial epidermis, the cells are polygonal and the anticlinal walls are straight to arched in *A. gracillima* (Fig. 6; Table 2), while in *A. delavayi*, the cells are irregular and the anticlinal walls are sinuate (Fig. 4; Table 2); on the abaxial epidermis, the wax ornamentation is flake-like in *A. gracillima* (Fig. 30; Table 3), while in *A. delavayi*, the wax ornamentation is invisible (Fig. 28; Table 3). The leaf epidermal features as well as the gross-morphological evidence, therefore, support the treatment of *A. gracillima* and *A. delavayi* as two distinct species.

Robinson (1898) described *Apios priceana* and noted that the corolla in this species was somewhat peculiar, with the standard having a thick, spongy, knot-like prolongation at apex. Based on this character, Robinson (1898) divided the genus *Apios* into two subgenera: subgen. *Tylosemium*, which includes only one species, *A. priceana*, and subgen. *Euapios*, which includes *A. americana*, *A. carnea*, *A. delavayi* and *A. fortunei*. Woods deemed that this subgeneric classification is not justified, as the same standard in all species of *Apios*, a prolongation at apex, although in some species the prolongation is greatly reduced (Woods, 1988). By examining more than 1000 *Apios* specimens, we also found that in all *Apios* species, the standard is connate and prolonged at apex. And our results of leaf epidermis, including shape of cells, pattern of anticlinal walls, cuticular membrane, wax ornamentation and type of stomatal apparatus, support Woods's view that there is no distinct epidermal apomorphy separating *A. priceana* from the remaining species of *Apios* (Tables 2, 3).

Apios and *Cochlianthus* are usually regarded as very closely related. They were grouped by Lackey (1977, 1981) as they share a spirally coiled style, a character which is not seen in any other groups in subtribe Erythrinae, tribe Phaseoleae. Woods (1988) recognized *Apios* and *Cochlianthus* as two separate but closely related genera based upon morphological criteria: in *Apios*, standard petal ovate to ovate-lanceolate, the auricle of the wing petal shorter than 2 mm, keel petal curved slightly, leaflets 5–7; in *Cochlianthus*, standard petal elliptic to rhombic, the auricle of the wing petal longer than 2 mm, keel petal curved strongly, leaflets 3. Based on both herbarium and field observation, we noticed that the leaves of *Apios* are primarily 5 or 7-foliolate, rarely 3-foliolate, almost glabrous to slightly velutinous on the veins and veinlets, while the leaves of *Cochlianthus* are exclusively trifoliolate, and densely tomentose on both surfaces. Leaf epidermal characters also showed some difference between these two genera: in *Apios*, anticlinal walls sinuate or straight to arched, wax ornamentation scale or gross flake-like, while in *Cochlianthus*, anticlinal walls sinuate and wax ornamentation condense flake-like (Tables 2, 3). Accordingly, we thought that *Apios* and *Cochlianthus* could be recognized as distinct and the relationship between the two genera may

not be so close as it was previously regarded.

The positions of *Apios* and *Cochlianthus* in subtribe Erythrinae, tribe Phaseoleae, are poorly understood. It is well established that Phaseoleae as currently circumscribed is not a monophyletic group and Erythrinae is also a highly polyphyletic assemblage of taxa (Lackey, 1981). Lackey (1977, 1981) suggested that subtribe Erythrinae is completely artificial and probably includes several independent lines with unknown nearest relatives, which is supported by DNA data (Bruneau et al., 1994; Wojciechowski et al., 2004). Based on the numbers of leaflets, Lackey held that some genera, including *Apios* and *Cochlianthus*, are suspected as imposters in the Phaseoleae. Therefore, additional evidence for interpreting the positions of *Apios* and *Cochlianthus* in tribe Phaseoleae, as well as the relationship between the two genera, is needed.

Acknowledgements We thank the curators of Herbarium of Institute of Botany, the Chinese Academy of Sciences (PE), Gray Herbarium of Harvard University (GH) and Herbarium of Royal Botanic Garden Edinburgh (E) for their generosity in providing specimens. We thank Dr. Gregory Kenicer (Royal Botanic Garden Edinburgh) for reviewing the manuscript. We also express our thanks to Mrs. WEN Jie, Mrs. YANG Zhi-Rong and Mrs. DU Yu-Fen for providing many helpful suggestions on our work and Mr. XIAO Yin-Hou and Mr. YANG Xue-Jian for the help during the process of developing films.

References

- Atchison E. 1949. Studies in the Leguminosae. IV. Chromosome numbers and geographical relationships of miscellaneous Leguminosae. *Journal of the Elisha Mitchell Scientific Society* 65: 118–122.
- Blackmon W J. 1986. Locating and growing groundnut. *Apios Tribune* 1: 5–7.
- Bruneau A, Anderson G J. 1988. Reproductive biology of diploid and triploid *Apios americana* (Leguminosae). *American Journal of Botany* 75: 1876–1883.
- Bruneau A, Anderson G J. 1994. To bee or not to bee?: The pollination biology of *Apios americana* (Leguminosae). *Plant Systematics and Evolution* 192: 147–149.
- Bruneau A, Doyle J J, Doyle J L. 1994. Phylogenetic relationships in Phaseoleae: evidence from chloroplast DNA restriction site characters. In: Crisp M, Doyle J J eds. *Advances in Legume Systematics 7: Phylogeny*. London: Royal Botanic Gardens, Kew. 309–330.
- De Candolle A P. 1825. *Prodromus systematis naturalis regni vegetabilis*. Paris: Sumptibus Sociorum Treuttel et Wurtz. 2: 390.
- Dilcher D L. 1974. Approaches to the identification of angiosperm leaf remains. *The Botanical Review* 40: 1–157.
- Duke J A. 1984. Properties of the groundnut. In: Hemenway D ed. *The International Permaculture Species Yearbook*. Orange, MA: Yankee Permaculture. 27–29.
- Haskin L L. 1929. “Nunas” and “noonas”: Indians love their roots and palefaces their flowers. *Nature Magazine* 14: 119.
- Hutchinson J. 1964. *The Genera of Flowering Plants (Angiospermae)*. Oxford, England: Oxford University Press.
- Joly S, Bruneau A. 2004. Evolution of triploidy in *Apios americana* (Leguminosae) revealed by genealogical analysis of histone H3-D gene. *Evolution* 58: 284–295.
- Krishnan H B. 1998. Identification of genistein, an anticarcinogenic compound, in the edible tubers of the American Groundnut (*Apios americana* Medikus). *Crop Science* 38: 1052–1056.
- Lackey J A. 1977. A revised classification of the tribe Phaseoleae (Leguminosae, Papilionoideae), and its relation to canavanine distribution. *Botanical Journal of Linnean Society* 74: 163–178.
- Lackey J A. 1981. Key to tribe of Papilionoideae, Phaseoleae. In: Polhill R M, Raven P H eds. *Advances in Legume Systematics. Part I*. London: Royal Botanic Gardens, Kew. 301–327.
- Lewis W H, Stripling H L, Ross R G. 1962. Chromosome numbers for some angiosperms of the southern United States and Mexico. *Rhodora* 64: 147–161.
- Li H-L (李惠林). 1952. Leguminosae. *Floristic Relationships Between Eastern Asia and Eastern North America*. Morris Arboretum, PA: University of Pennsylvania. 390–391.
- Li S-G (李树刚). 1995. Leguminosae. In: *Flora Reipublicae Popularis Sinicae (中国植物志)*. Beijing: Science

- Press. 41: 199–206.
- Robinson B L. 1898. A new species of *Apios* from Kentucky. The Botanical Gazette 25: 450–453.
- Safford W E. 1925. The potato of romance and of reality. The Journal of Heredity 16: 113–126.
- Seabrook J E A, Dionne L A. 1976. Studies on the genus *Apios*. I. Chromosome number and distribution of *Apios americana* and *A. priceana*. Canadian Journal of Botany 54: 2567–2572.
- Stace C A. 1984. The taxonomic importance of the leaf surface. In: Heywood V H, Moore D M eds. Current Concepts in Plant Taxonomy. London: Academic Press. 67–94.
- Vietmeyer N. 1986. The wild groundnut. The next potato? *Apios* Tribune 1: 2–5.
- Walker W C. 1939. A string of new potatoes. Nature Magazine 32: 526–527.
- Walter W M, Croom E M Jr., Catignant G L, Thresher W C. 1986. Compositional study of *Apios priceana* tubers. Journal of Agricultural and Food Chemistry 4: 39–41.
- Westerkamp C, Paul H. 1993. *Apios americana*, a fly-pollinated papilionaceous flower? Plant Systematics and Evolution 187: 135–144.
- Wilkinson H P. 1979. The plant surface (mainly leaf). In: Metcalfe C R, Chalk L eds. Anatomy of the Dicotyledons. 2nd ed. Oxford: Clarendon Press. 1: 97–165.
- Wojciechowski M F, Lavin M, Sanderson M J. 2004. A phylogeny of legumes (Leguminosae) based on analysis of the plastid *matK* gene resolves many well-supported subclades within the family. American Journal of Botany 91: 1846–1862.
- Woods M. 1988. A revision of *Apios* and *Cochlianthus* (Leguminosae). Ph.D. Dissertation. Carbondale, IL: Southern Illinois University.

土圞儿属和旋花豆属(豆科)的叶表皮特征 及其系统学意义

^{1,2}任 勃 ¹朱相云* ³姜彦成*

¹ (中国科学院植物研究所 北京 100093)

² (中国科学院研究生院 北京 100049)

³ (新疆大学生命科学技术学院 新疆 830046)

摘要 应用光学显微镜和扫描电子显微镜观察了豆科 Leguminosae 土圞儿属 *Apios* 和旋花豆属 *Cochlianthus* 植物的叶表皮特征。在光学显微镜下,大多数种类的叶表皮细胞形状(表面观)为不规则型,垂周壁式样为浅波状或波状,只有一个种——*Apios gracillima* Dunn 叶表皮细胞为多边形,垂周壁式样为较平直。在扫描电子显微镜下,多数种类的叶片蜡质纹饰通常为片状或颗粒状,一些种叶表皮未见蜡质纹饰;气孔器外拱盖内缘通常为浅波状或近平滑。在这两个属内,上述叶表皮特征在物种内较稳定,因此对于解决属内种间和属间关系有一定的系统学意义。本研究得出以下结论: (1) *A. gracillima* 叶的上表皮细胞为多边形,垂周壁平直或弧形, *A. delavayi* Franch. 叶的上表皮细胞为不规则形,垂周壁波状,二者存在明显差异,因此 *A. gracillima* 应处理为一独立的种,而不是 *A. delavayi* 下的一个变种; (2) *A. priceana* B. L. Rob. 在叶表皮细胞形状、垂周壁式样、气孔器类型和蜡质纹饰形态等方面与土圞儿属中其他种不存在显著区别,因此土圞儿属内两个亚属的划分是不合理的; (3) 土圞儿属多为5或7小叶,无毛或近无毛,叶表皮细胞垂周壁波状或近平直,而旋花豆属3小叶,密被柔毛,叶表皮细胞垂周壁浅波状,本文支持它们为两个独立的属的分类处理。

关键词 豆科; 土圞儿属; 旋花豆属; 叶表皮; 系统学意义